产品名称：**TAK 632**
产品别名：**TAK-632**

生物活性：

Description

TAK-632 is a potent pan-RAF inhibitor with IC_{50} of 1.4, 2.4 and 8.3 nM for CRAF, $BRAF^{V600E}$, $BRAF^{WT}$, respectively.

IC_{50} & Target

c-Raf	Braf	Aurora B	PDGFRβ	PDGFRα
1.4 nM (IC_{50})	8.3 nM (IC_{50})	66 nM (IC_{50})	120 nM (IC_{50})	610 nM (IC_{50})
FGFR3	TIE2	IKKβ	CDK1	CDK2
280 nM (IC_{50})	740 nM (IC_{50})	3700 nM (IC_{50})	790 nM (IC_{50})	580 nM (IC_{50})
p38α	GSK3β	MEK1		
600 nM (IC_{50})	500 nM (IC_{50})	3700 nM (IC_{50})		

In Vitro

TAK-632 inhibits PDGFRβ, FGFR3, GSK3β, CDK2, P38α, PDGFRα, TIE2, and CDK1 with a range of IC50 values from 120-790 nM. CHK1, IKKβ, and MEK1 are inhibited over an IC50 range of 1400-1700 nM. With 1 h of preincubation time, TAK-632 inhibits BRAF and CRAF in an ATP competitive manner (at low ATP concentrations BRAF IC50: 15 nM; CRAF: 8.1 nM). The respective biochemical activity of TAK-632 against BRAF and CRAF reduces to IC50 values of 58 nM and 62 nM at high ATP concentrations.TAK-632 demonstrates strong inhibition of pMEK and pERK in HMVII cells with IC50 values of 49 nM and 50 nM, respectively[1]. TAK-632 shows strong antiproliferative effects both in A375 and SK-MEL-2 cells (GI50 of 40-190 nM in A375 cells and GI50 of 190-250 nM in SK-MEL-2 cells)[2].

In Vivo

TAK-632 demonstrates dramatically improved solubility (740 μg/mL) in pH 6.8 phosphate buffer and exhibits significant oral absorption (at a dose of 25 mg/kg, AUC, 32.47 μg h/mL; F, 51.7%) in rats. In a dog PK study, 10 mg/kg administration of TAK-632 also shows superior oral bioavailability (F: 108%).Oral single administration of TAK-632 inhibits pERK in tumors at 8 h after its administration over a dose range of 1.9-24.1 mg/kg. In particular, 9.7-24.1 mg/kg dosing with TAK-632 strongly inhibits pERK levels to 11% of the control. TAK-632 exhibits dose-dependent antitumor efficacy without severe body weight reduction over a dose range of 3.9-24.1 mg/kg. Significant tumor regression is observed at 9.7 mg/kg and 24.1 mg/kg (T/C=−2.1% and −12.1%, respectively)[1]. TAK-632 exhibits potent antitumor efficacy when orally administered at 60 mg/kg once daily (T/C=37%, P<0.001) or at 120 mg/kg once daily (T/C=29%, P<0.001) for 21 days without severe toxicity in NRAS-mutant melanoma using a SK-MEL-2 xenograft model[2].

In Vitro:

DMSO： 100 mg/mL (180.34 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	1.8034 mL	9.0168 mL	18.0336 mL
	5 mM	0.3607 mL	1.8034 mL	3.6067 mL
	10 mM	0.1803 mL	0.9017 mL	1.8034 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

Solvent&Solubility	请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 **In Vitro** 方式配制澄清的储备液，再依次添加助溶剂： ——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶 1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline Solubility: 2.5 mg/mL (4.51 mM); Suspended solution; Need ultrasonic 此方案可获得 2.5 mg/mL (4.51 mM)的均匀悬浊液，悬浊液可用于口服和腹腔注射。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。 2.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (4.51 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (4.51 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Okaniwa M, et al. Discovery of a selective kinase inhibitor (TAK-632) targeting pan-RAF inhibition: design, synthesis, and biological evaluation of C-7-substituted 1,3-benzothiazole derivatives. J Med Chem. 2013 Aug 22;56(16):6478-94. [2]. Nakamura A, et al. Antitumor activity of the selective pan-RAF inhibitor TAK-632 in BRAF inhibitor-resistant melanoma. Cancer Res. 2013 Oct 11.

实验参考：

Cell Assay	Cell viability is assessed (3 replicates) using the Sulforhodamine B assay or by the CellTiter-Glo luminescent cell viability assay. The concentrations of TAK-632 that produced 50% growth inhibition (GI_{50}) are calculated using PCP software. The combination index (CI) is calculated using CalcuSyn software. To investigate the antiproliferative activity of TAK-632, we performed proliferation assays in various cell lines harboring mutated BRAF, NRAS, or KRAS. HMV-II, SK-MEL-2, or A375 cells are cotreated with TAK-632 and TAK-733 at the indicated concentrations for 72 hours. Cell viability is measured. The CI value at EC_{50} is calculated. A375 cells stably expressing $NRAS^{Q61K}$ or ΔN-BRAF are cotreated with TAK-632 and TAK-733 at the indicated concentrations for 72 hours. Cell viability is measured. The CI value at EC_{50} is calculated[2].
Animal Administration	Mice[2] The xenograft-implanted nude mice are used. Mice bearing SK-MEL-2 xenografts are treated once daily for 21 consecutive days with vehicle or TAK-632 at the indicated concentrations (10 mice per each treatment group). Day 0 indicates the beginning of treatment. Tumors are measured twice a week. Mice bearing SK-MEL-2 xenografts are treated once daily (QD) for 3 days with vehicle, TAK-632 at 60 mg/kg (60 mpk), or TAK-632 at 120 mg/kg (120 mpk). Tumor xenografts are obtained at indicated time points after the final treatment and analyzed by Western blot analysis. Individual blots with dividing lines are combined from a single electrophoresis gel. Bars represent densitometric analysis of phospho-ERK, normalized to vehicle-treated control (mean±SD).
Kinase Assay	Immunoprecipitated BRAF or CRAF is incubated with recombinant inactive MEK (K97R) at 30°C for 30 minutes in kinase reaction buffer containing ATP/Mg^{2+}. RAS/RAF wild-type (A431, CsFb, and HeLa), KRAS-mutant (A549, HCT-116, and MIA PaCa-2), and NRAS-mutant melanoma (GAK,

	HMV-II, and SK-MEL-2) cells are treated with TAK-632 (0, 0.32, 1.6, 8, 40, 200, 1000 and 5000 nM) at the indicated concentrations for 2 hours. Cell lysates are analyzed by Western blot analysis[2].
References	[1]. Okaniwa M, et al. Discovery of a selective kinase inhibitor (TAK-632) targeting pan-RAF inhibition: design, synthesis, and biological evaluation of C-7-substituted 1,3-benzothiazole derivatives. J Med Chem. 2013 Aug 22;56(16):6478-94. [2]. Nakamura A, et al. Antitumor activity of the selective pan-RAF inhibitor TAK-632 in BRAF inhibitor-resistant melanoma. Cancer Res. 2013 Oct 11.